

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Fandke et al.

Art Unit: Unassigned

Application No. Unassigned
(U.S. National Phase of PCT/EP00/08808)

Examiner: Unassigned

Filed: March 20, 2002

For: METHOD AND NUCLEIC ACIDS FOR
THE DETECTION OF
MICROORGANISMS RELEVANT TO
BREWING

AMENDMENTS TO CLAIMS**MADE VIA PRELIMINARY AMENDMENT**

[1. Method for the detection of microorganisms relevant to brewing in a sample, which comprises the following steps:

- (a) bringing the sample into contact with a combination of at least two first nucleic acid molecules (primers), which hybridise with a region of a microbial nucleic acid conserved in microorganisms relevant to brewing;
- (b) amplification of the microbial nucleic acid or a portion thereof to produce at least one amplification fragment;
- (c) bringing the amplification fragments obtained in step (b) into contact with at least one second nucleic acid molecule (probe), which specifically hybridises with at least one amplification fragment that comprises a sequence of the microbial nucleic acid specific for all microorganisms relevant to brewing or for one or several families, genera or species of microorganisms relevant to brewing;
- (d) detection of at least one hybrid nucleic acid which consists of an amplification fragment and a second nucleic acid molecule introduced in step (c).]

[2. Method according to Claim 1, characterised in that the amplification comprises a polymerase chain reaction (PCR).]

[3. Method according to Claim 1, characterised in that the amplification comprises a ligase chain reaction.]

[4. Method according to Claim 1, characterised in that the amplification comprises an isothermal nucleic acid amplification.]

[5. Method according to one of Claims 1 to 4, characterised in that the second nucleic acid molecule is modified or labelled to produce a detectable signal, the modification or labelling being selected from (i) radioactive groups, (ii) coloured groups, (iii) fluorescent groups, (iv) groups for immobilisation on a solid phase and (v) groups which allow an indirect or direct reaction, particularly by means of antibodies, antigens, enzymes and/or substances with affinity for enzymes or enzyme complexes.]

[6. Method according to one of the preceding Claims, characterised in that the first nucleic acid molecule and/or the second nucleic acid molecule are at least 10 nucleotides, preferably 15-30 nucleotides long.]

[7. Method according to one of the preceding Claims, characterised in that the first nucleic acid molecule and/or the second nucleic acid molecule is modified in that up to 20% of the nucleotides in 10 consecutive nucleotides, in particular 1 or 2 nucleotides from the block of 10 are replaced by nucleotides which do not naturally occur in bacteria.]

[8. Method according to one of the preceding Claims, characterised in that the conserved region occurs in the genome section which contains the bacterial 23 S and 5 S genes.]

[9. Nucleic acid molecule as probe and/or primer for the detection of microorganisms relevant to brewing, said nucleic acid molecule being selected from:

- (i) a nucleic acid with a sequence according to SEQ ID NO 1-107 or a fragment thereof at least 10, preferably 15-30 nucleotides long;
- (ii) a nucleic acid which specifically hybridises with a nucleic acid according to (i);
- (iii) a nucleic acid which is at least 70%, preferably at least 90%, identical with a nucleic acid according to (i) or (ii), or
- (iv) a nucleic acid which is complementary to a nucleic acid according to (i) to (iii).]

[10. Nucleic acid molecule according to Claim 9, characterised in that it is a DNA or an RNA.]

[11. Nucleic acid molecule according to Claim 9, characterised in that it is a PNA.]

[12. Nucleic acid molecule according to Claim 9 to 11, characterised in that up to 20% of the nucleotides in 10 consecutive nucleotides, in particular 1 or 2 nucleotides from the block of 10 are replaced by nucleotides which do not occur naturally in bacteria.]

[13. Combination of at least two nucleic acid molecules, said combination being selected from:

- (1) a combination of at least two nucleic acid molecules according to one of Claims 9 to 12, and
- (2) a combination which comprises at least one nucleic acid molecule with a sequence according to one of the SEQ ID NO 40 to 47 and at least one nucleic acid molecule with a sequence according to SEQ ID NO 48-54 or SEQ ID NO 55-59 or SEQ ID NO 60-72.]

[14. Kit containing a nucleic acid molecule according to one of Claims 9 to 12 and/or a combination according to Claim 13.]

[15. Method according to one of Claims 1 to 8, characterised in that in step (a) a combination of at least two nucleic acid molecules according to Claim 13 is used.]

[16. Method according to one of Claims 1 to 8 and 15, characterised in that as second nucleic acid molecule (probe) at least one nucleic acid molecule according to one of Claims 9 to 12 is used.]

[17. Method according to Claim 16, characterised in that as second nucleic acid molecule (probe) at least one nucleic acid molecule with a sequence according to one of SEQ ID NO 35 to 39 or 98-107 is used.]

[18. Method according to Claim 16 or 17, characterised in that as second or further nucleic acid molecule (probe) at least one nucleic acid molecule with a sequence according to one of SEQ ID NO 21 to 34 or SEQ ID NO 73-97 is used.]

[19. Use of a nucleic acid molecule according to one of Claims 9 to 12 and/or a combination according to Claim 13 for the detection of bacteria relevant to brewing.]

[20. Use of a nucleic acid molecule according to one of Claims 9 to 12 for the identification and/or characterisation of bacteria relevant to brewing.]

[21. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 35 or SEQ ID NO 86 for the detection and/or for the identification and/or characterisation of bacteria of the genus *Pediococcus*.]

[22. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 36 or SEQ ID NO 104 for the detection and/or for the identification and/or characterisation of bacteria of the genus *Pectinatus*.]

[23. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 37 or SEQ ID NO 107 for the detection and/or for the identification and/or characterisation of bacteria of the genus *Megasphaera*.]

[24. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 38 or SEQ ID NO 105 for the detection and/or for the identification and/or characterisation of bacteria of the genus *Selenomonas*.]

[25. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 39 or SEQ ID NO 106 for the detection and/or for the identification and/or characterisation of bacteria of the genus *Zymophilus*.]

[26. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 1, SEQ ID NO 21 or SEQ ID NO 73-74 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus brevis*.]

[27. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 2, SEQ ID NO 22 or SEQ ID NO 75-76 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus lindneri*.]

[28. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 3, SEQ ID NO 23 or SEQ ID NO 77-79 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus casei*.]

[29. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 23 or SEQ ID NO 79-81 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus paracasei* ssp. *paracasei*.]

[30. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 6, SEQ ID NO 24 or SEQ ID NO 82 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus coryniformis* ssp. *coryniformis*.]

[31. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 7, SEQ ID NO 24 or SEQ ID NO 82 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus coryniformis* ssp. *torquens*.]

[32. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 8, SEQ ID NO 25 or SEQ ID NO 83 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus curvatus*.]

[33. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 9, SEQ ID NO 26, SEQ ID NO 84 or SEQ ID NO 86 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Pediococcus damnosus*.]

[34. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 85 or SEQ ID NO 86 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Pediococcus inopinatus*.]

[35. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 11, SEQ ID NO 28 or SEQ ID NO 87-88 or a fragment thereof with at least 10, preferably 15-30 nucleo-tides for the detection and/or for the identification and/or characterisation of bacteria of the species *Pectinatus cerevisiiphilus*.]

[36. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 12, SEQ ID NO 29 or SEQ ID NO 89-90 or a fragment thereof with at least 10, preferably 15-30 nucleo-tides for the detection and/or for the identification and/or characterisation of bacteria of the species *Pectinatus frisingiensis*.]

[37. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 13, SEQ ID NO 14, SEQ ID NO 30 or SEQ ID NO 91-93 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the strain *Pectinatus sp. DSM20764*.]

[38. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 15, SEQ ID NO 16, SEQ ID NO 31 or SEQ ID NO 97 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Megasphaera cerevisiae*.]

[39. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 17, SEQ ID NO 18, SEQ ID NO 32 or SEQ ID NO 94 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Selenomonas lacticifex*.]

[40. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 19, SEQ ID NO 33 or SEQ ID NO 95 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Zymophilus raffinosivorans*.]

[41. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 20, SEQ ID NO 34 or SEQ ID NO 96 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Zymophilus paucivorans*.]

42. Method for the detection of a microorganism relevant to brewing in a sample, which comprises the following steps:

- (a) bringing the sample into contact with a combination of at least two first nucleic acid molecules (primers), which hybridise with a region of a microbial nucleic acid conserved in microorganisms relevant to brewing;
- (b) amplification of the microbial nucleic acid or a portion thereof to produce at least one amplification fragment;
- (c) bringing the amplification fragments obtained in step (b) into contact with at least one second nucleic acid molecule (probe), which specifically hybridises with at least one amplification fragment that comprises a sequence of the microbial nucleic acid specific for all microorganisms relevant to brewing or for one or several families, genera or species of microorganisms relevant to brewing; and
- (d) detection of at least one hybrid nucleic acid which consists of an amplification fragment and a second nucleic acid molecule introduced in step (c), whereupon a microorganism relevant to brewing is detected in a sample.

43. Method according to Claim 42, characterised in that as second nucleic acid molecule (probe) at least one nucleic acid molecule, selected from

- (i) a nucleic acid with a sequence according to SEQ ID NOS: 1-107 or a fragment thereof at least 10 nucleotides long;
- (ii) a nucleic acid which specifically hybridises with a nucleic acid according to (i);
- (iii) a nucleic acid which is at least 70% identical with a nucleic acid according to (i) or (ii), and
- (iv) a nucleic acid which is complementary to a nucleic acid according to (i) to (iii).

44. Method according to Claim 43, characterised in that as second nucleic acid molecule (probe) at least one nucleic acid molecule with a sequence according to one of SEQ ID NOS: 35-39 or 98-107 is used.

45. Method according to Claim 43, characterised in that as second or further nucleic acid molecule (probe) at least one nucleic acid molecule with a sequence according to one of SEQ ID NOS: 21-34 or SEQ ID NO 73-97 is used.

46. Method according to Claim 42, characterised in that in step (a) a combination of at least two nucleic acid molecules is used, combination being selected from

- (i) a nucleic acid with a sequence according to SEQ ID NOS: 1-107 or a fragment thereof at least 10 nucleotides long;
- (ii) a nucleic acid which specifically hybridises with a nucleic acid according to (i);
- (iii) a nucleic acid which is at least 70% identical with a nucleic acid according to (i) or (ii),
- (iv) a nucleic acid which is complementary to a nucleic acid according to (i) to (iii), and
- (v) a combination which comprises at least one nucleic acid molecule with a sequence according to one of the SEQ ID NOS: 40-47 and at least one nucleic acid molecule with a sequence according to SEQ ID NOS: 48-54, SEQ ID NOS: 55-59 or SEQ ID NOS: 60-72.

47. Method according to Claim 46, characterised in that as second nucleic acid molecule (probe) at least one nucleic acid molecule according to (i)-(iv) is used.

48. Method according to Claim 47, characterised in that as second nucleic acid molecule (probe) at least one nucleic acid molecule with a sequence according to one of SEQ ID NOS: 35-39 or 98-107 is used.

49. Method according to Claim 47, characterised in that as second or further nucleic acid molecule (probe) at least one nucleic acid molecule with a sequence according to one of SEQ ID NOS: 21-34 or SEQ ID NO 73-97 is used.

50. Method according to Claim 42, characterised in that the amplification comprises a polymerase chain reaction (PCR).

51. Method according to Claim 42, characterised in that the amplification comprises a ligase chain reaction.

52. Method according to Claim 42, characterised in that the amplification comprises an isothermal nucleic acid amplification.

53. Method according to Claim 42, characterised in that the second nucleic acid molecule is modified or labelled to produce a detectable signal, the modification or labelling being selected from (i) radioactive groups, (ii) coloured groups, (iii) fluorescent groups, (iv) groups for immobilisation on a solid phase and (v) groups which allow an indirect or direct reaction, particularly by means of antibodies, antigens, enzymes and/or substances with affinity for enzymes or enzyme complexes.

54. Method according to Claim 42, characterised in that the first nucleic acid molecule and/or the second nucleic acid molecule are at least 10 nucleotides long.

55. Method according to Claim 54, characterized in that the first nucleic acid molecule and/or the second nucleic acid molecule are at least 15-30 nucleotides long.

56. Method according to Claim 42, characterised in that the first nucleic acid molecule and/or the second nucleic acid molecule is modified in that up to 20% of the nucleotides in 10 consecutive nucleotides are replaced by nucleotides which do not naturally occur in bacteria.

57. Method according to Claim 42, characterised in that the conserved region occurs in the genome section which contains the bacterial 23 S and 5 S genes.

58. Nucleic acid molecule as probe and/or primer for the detection of microorganisms relevant to brewing, said nucleic acid molecule being selected from:

- (i) a nucleic acid with a sequence according to SEQ ID NOS: 1-107 or a fragment thereof at least 10 nucleotides long;
- (ii) a nucleic acid which specifically hybridises with a nucleic acid according to (i);
- (iii) a nucleic acid which is at least 70% identical with a nucleic acid according to (i) or (ii), and
- (iv) a nucleic acid which is complementary to a nucleic acid according to (i) to (iii).

59. Nucleic acid molecule of Claim 58, wherein the nucleic acid of (i) is at least 15-30 nucleotides long and the nucleic acid of (iii) is at least 90% identical with a nucleic acid according to (i) or (ii).

60. Nucleic acid molecule according to Claim 58, characterised in that it is a DNA or an RNA.

61. Nucleic acid molecule according to Claim 58, characterised in that it is a PNA.

62. Nucleic acid molecule according to Claim 58, characterised in that up to 20% of the nucleotides in 10 consecutive nucleotides are replaced by nucleotides which do not occur naturally in bacteria.

63. Combination of at least two nucleic acid molecules, said combination being selected from:

- (1) a combination of at least two nucleic acid molecules according to Claim 58, and
- (2) a combination which comprises at least one nucleic acid molecule with a sequence according to one of the SEQ ID NOS: 40-47 and at least one nucleic acid molecule with a sequence according to SEQ ID NOS: 48-54, SEQ ID NOS: 55-59 or SEQ ID NOS: 60-72.